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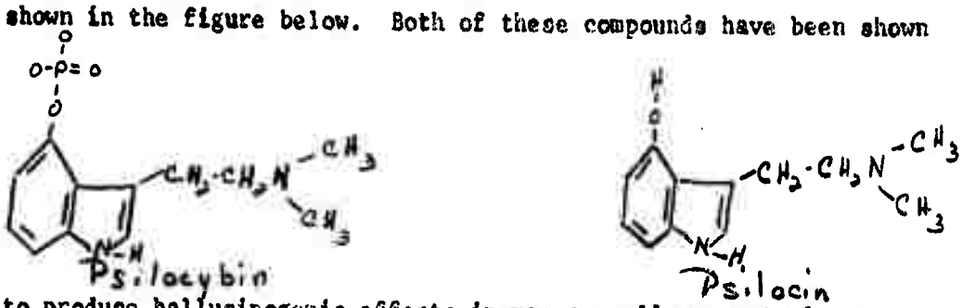
SOME BIOCHEMICAL STUDIES ON PSILOCYBIN AND PSILOCIN

In this presentation I would like to discuss some of our studies on two of the indoleamines recently identified in the mushroom, Psilocybe mexicana Heim. These are psilocybin (4-phosphoryl-N,N-dimethyltryptamine) and its 4-hydroxy analogue, psilocin.[3,4] Their chemical structures are shown in the figure below. Both of these compounds have been shown



to produce hallucinogenic effects in man as well as central nervous system actions in the experimental animal.

Our interest in these compounds first arose when we observed that psilocybin could be enzymatically dephosphorylated by purified preparations of intestinal phosphatase as well as by human serum alkaline phosphatase.[5] This was expected since psilocybin resembles chemically other arylphosphates which are excellent substrates of the enzyme. These initial experiments suggested the possibility that psilocybin might also undergo rapid dephosphorylation in the body with the subsequent liberation of psilocin, and that the pharmacologic activity of psilocybin might be produced by this 4-hydroxy congener. Such a phenomenon was entirely possible, for upon determining the solubilities of the two compounds in organic solvents as compared to that in aqueous solution at pH 7.4, it was found that psilocin indicated a much higher lipid solubility than psilocybin. Since lipid solubility plays such an important role in the passage of drug molecules through the blood-brain barrier, it would appear that psilocin would be more effectively taken up by the brain tissue.

Before proceeding to the intact animal we continued our earlier enzyme studies by employing various tissue preparations.[6] The rat kidney homogenate, known to possess high alkaline phosphatase activity, was employed as the source of the enzyme. Most of the work was carried out at pH 8.8 because of the high pH optimum of alkaline phosphatase. Unlike the experiments with the purified intestinal phosphatase, however, the incubation of psilocybin with kidney homogenates resulted in the rapid formation of a blue colored product. Dephosphorylation had occurred, but the psilocin which was liberated could not be measured accurately because of its rapid breakdown to the blue product. It was immediately evident that in the kidney homogenate another process was present which was responsible for the rapid degradation of psilocin. Preliminary investigation indicated this process to be the action of an oxidative enzyme, for it was inhibited by KCN and by anaerobic conditions. It was not monoamine oxidase (MAO) for, among other things, the usual inhibitors of this enzyme was ineffective in preventing the oxidation process. In the kidney homogenate, therefore, two enzymes were active in metabolizing the 4-substituted indoleamines; namely, the alkaline phosphatase which acted to dephosphorylate psilocybin, resulting in the liberation of psilocin, and the oxidation of psilocin, presumably at the 4-OH position, by an oxidative enzyme. Extensive studies of these two systems including pH optima, the actions of various inhibitors, organ and tissue distribution, as well as species differences were undertaken. From the characteristics of this system we concluded that the oxidation of psilocin was carried out by the cytochrome oxidase enzyme. As final support we were able to demonstrate the oxidation of psilocin with a purified preparation of cytochrome oxidase in the presence of cytochrome c. Again, under these conditions, the usual blue colored product was formed with the concommitant

utilization of oxygen and disappearance of psilocin. The nature of the blue colored product is not known, but it is possibly a quinone-type structure, for such compounds are often highly colored. Blaschko and Levine[1,2] recently described the oxidation of psilocin and other hydroxyindoles by preparations of the gill plates of the Mytilus edulis and attributed this to the action of a hydroxyindole oxidase found in this species of mollusc. The oxidation of psilocin with these preparations also resulted in a blue colored product, probably the same as found in our experiments. The enzyme responsible for this oxidation, however, appears to be quite different.

Digressing a few moments from the subject of the metabolism of 4-substituted indoleamines I might mention here some interesting results obtained when serotonin (5-hydroxytryptamine, 5HT) instead of psilocin was employed as the substrate. Incubation of serotonin with rat liver, brain, kidney and heart homogenates over a pH range of 7.4 to 9.5 resulted in considerately different degrees of metabolism, expecially in the presence of inhibitor substances. We may assume that rat liver and brain preparations oxidized serotonin primarily via the monoamine oxidase pathways since the usual MAO inhibitors effectively blocked substrate disappearance over the entire range of pH levels. With the heart and kidney tissues a very different picture was observed. At pH 7.4 the metabolism of serotonin appeared to proceed mainly by the MAO, again as evidenced by its inhibition by the well known inhibitors, phenylisopropylhydrazine (PIH) and tranylcypromine (2-phenylcyclopropylamine, SKF-385). Oxidation of the substrate did not decrease with increasing pH, but its inhibition by the usual MAO inhibitors became less and less active, and at pH 9.5 they were completely ineffective in blocking the oxidation of 5HT. KCN, on the other hand, became an active

inhibitor; however, this occurred only at the higher pH ranges and only when the MAO inhibitors were ineffective.

In these instances it was concluded that the alteration of pH caused the activation of a second enzyme system which was acting on the 5-OH portion of the molecule. As seen with psilocin the oxidation of the -OH group of serotonin appeared to be by way of the action of cytochrome oxidase. Again cytochrome c enhanced the oxidation process. Recent studies indicated serotonin to be an effective substrate of the purified enzyme and cytochrome c, although not as efficient as the 4-hydroxylated compounds. Under the conditions described here, then serotonin oxidation can occur by two enzymatic pathways. First, as seen in the liver and brain over a wide range of pH, and in heart and kidney at pH 7.4, is the oxidative deamination by MAO. The second route of breakdown occurs via the cytochrome system which acts on the -OH group of the indole ring, and this is demonstrable with kidney and heart preparations at the higher pH levels.

That MAO is important in the metabolism of serotonin in the intact animal is well established. The importance of the cytochrome system as a physiological mechanism of serotonin degradation is rather dubious, especially with the high pH optimum and the fact that metabolites of such oxidation products have not been identified. At best it may play a minor role in the breakdown of hydroxylated indoleamines.

Returning to the studies of the 4-substituted indole compounds, we preceeded to determine whether the dephosphorylation of psilocybin could be demonstrated in the intact animal.[8] Mice were given 100 mg/kg psilocybin, sacrificed at periodic intervals and the livers, kidneys and brains analyzed for psilocin. A rapid appearance of psilocin occurred in both liver and kidney, while accumulation in brain was considerably

slower. Observation of the behavior of mice thus treated also revealed marked pharmacological signs such as exophthalmus, pilocerection, motor incoordination and depression. This, of course, did not prove that the psilocin which was liberated from the dephosphorylation of psilocybin was the active agent in producing these effects. There was still the possibility that psilocybin itself might be the active agent. In order to resolve this problem we felt that the use of an inhibitor of alkaline phosphatase was warranted. By blocking the dephosphorylating reaction it would be possible to prevent the liberation of psilocin, and, if psilocin were the active product, the central nervous system effects as well. Ideally, an irreversible inhibitor such as iproniazid exerts on MAO and DFP on acetylcholinesterase would have been desirable for this purpose. Such agents, however, which are useful and effective in vivo are not known for alkaline phosphatase. We therefore approached the problem by employing another substrate, sodium β-glycerophosphate, with the hope that this would compete with psilocybin, thus decreasing its dephosphorylation to psilocin. Preliminary experiments showed sodium β-glycerophosphate and its dephosphorylated product, glycerol, to be non-toxic even at high doses, and no apparent pharmacologic actions were produced in the intact mouse.

The administration of psilocybin to mice pretreated with the β-glycerophosphate resulted in a considerably lower accumulation of psilocin in those tissues investigated. Not only the amount but also the rate of psilocin appearance was greatly attenuated as compared to control animals. Observation of the behavioral effects also showed a much less exaggerated picture, suggesting that the decrease psilocin liberation was responsible for the attenuated response.

These experiments stongly supported the hypothesis that in the intact mouse the central nervous system action of psilocybin was being produced by its dephosphorylated analogue psilocin. One argument still remained, however, and this was the possibility of the sodium -glycerophosphate acting not only as a competitive substrate but also as a pharmacological antagonist of psilocybin or psilocin, thus decreasing the normal actions of these indoleamines. A final series of experiments was undertaken to rule out such a possibility. Control and glycerophosphate pretreated mice were given injections of psilocin and their tissue levels of this compound were measured. Changes in behavior were also observed closely. In all tissues investigated the levels of psilocin did not differ between control and glycerophosphate-pretreated mice, nor were there any observable differences in the behavioral effects between the two groups.

These results, therefore, established the relationship between the intensity of the central nervous system actions and the rate of appearance of psilocin in brain and other tissues after the administration of psilocybin. They also provide another example where a pharmacologically active agent is produced by the action of enzymes on an inactive compound.

SUMMARY

This paper has reviewed some of our recent biochemical studies with the 4-substituted indoleamines, psilocybin and psilocin. Both *in vivo* and *in vitro* psilocybin is rapidly dephosphorylated to liberate its 4-OH analogue, psilocin. The possibility that psilocin may be the pharmacologically active agent after psilocybin administration is discussed.

Under *in vitro* conditions the heart and kidney tissues of various animals are capable of oxidizing psilocin to a blue colored product. This oxidation process is catalyzed by the cytochrome c - cytochrome oxidase pathway, and it appears to be an action at the -OH group on the indole ring.

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